(12) UK Patent Application (19) GB (11) 2 338 568 (13) A

(43) Date of A Publication 22.12.1999

(21) Application No 9914002.2

(22) Date of Filing 17.06.1999

(30) Priority Data
(31) 4254 (32) 19.06.1998 (33) AU
(31) 5207 (32) 12.08.1998
(31) 7160 (32) 17.11.1998

(51) INT CL[6]
G02B 23/26

(52) UK CL (Edition Q)
G2J JB7R4

(56) Documents Cited
None

(58) Field of Search
UK CL (Edition Q) G2J JB7R4
INT CL[6] G02B
Online: WPI, EPODOC, JAPIO

(71) Applicant(s)
Optiscan Pty Ltd
(Incorporated in Australia - Victoria)
27 Normanby Road, Notting Hill, Victoria 3168, Australia

(72) Inventor(s)
Martin Russell Harris

(74) Agent and/or Address for Service
Eric Potter Clarkson
Park View House, 58 The Ropewalk, NOTTINGHAM, NG1 5DD, United Kingdom

(54) Abstract Title
Endoscopes and microscopes

(57) An endoscope or microscope 10 includes an optic fibre for transmitting a pulsed main beam 14 along at least a portion of an optical path between a beam source 12 and a sample 44, and a dispersing means 28, 30 whereby dispersion occurring in the optic fibre 20 is reversed by dispersion produced by the dispersing means.

An endoscope or microscope with a head, the endoscope or microscope includes a suction or partial vacuum source located near the head, a light collecting means for collecting light from a sample, and a sample receiving means coupled to the suction source for receiving the sample when the sample is urged thereto by the suction source, wherein the suction source is used to urge the sample towards the receiving means, thereby deforming the sample so that the light collecting means can collect some of light emitted by the sample away from the head.

An endoscope or microscope has means for synchronising switching of illumination on and off and acquisition periods.

An endoscope or microscope has a photodetector and a filter to reduce detection of ambient illumination.

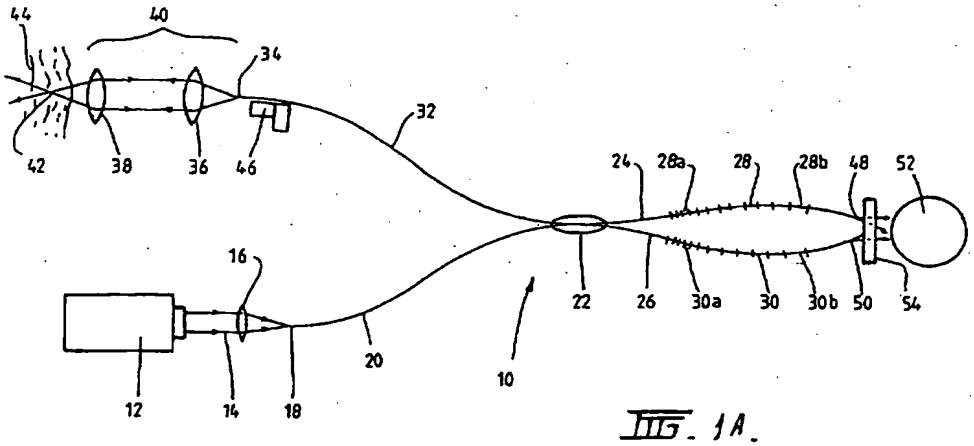

Fig. 1A.

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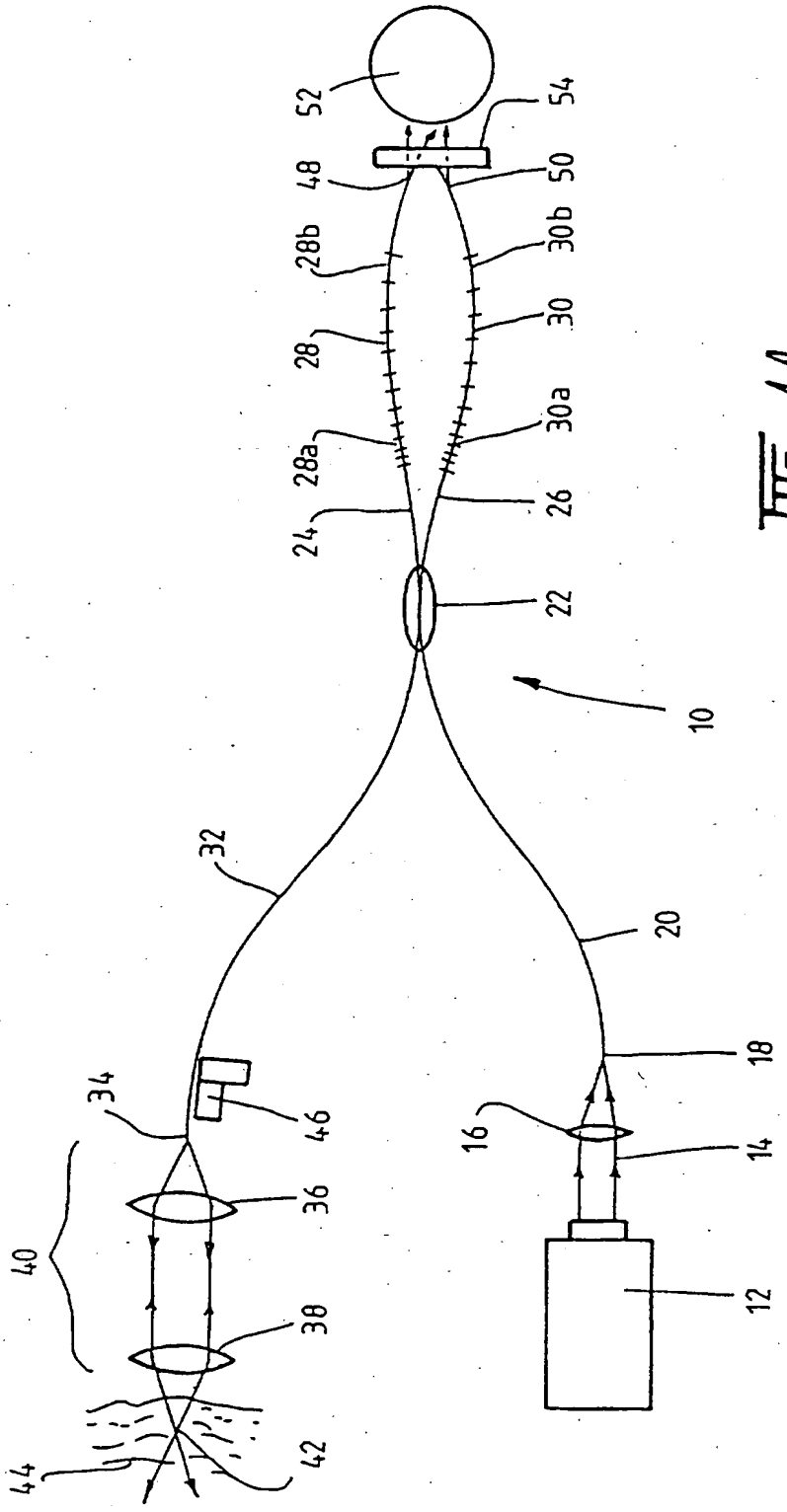
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FIG. 1A.

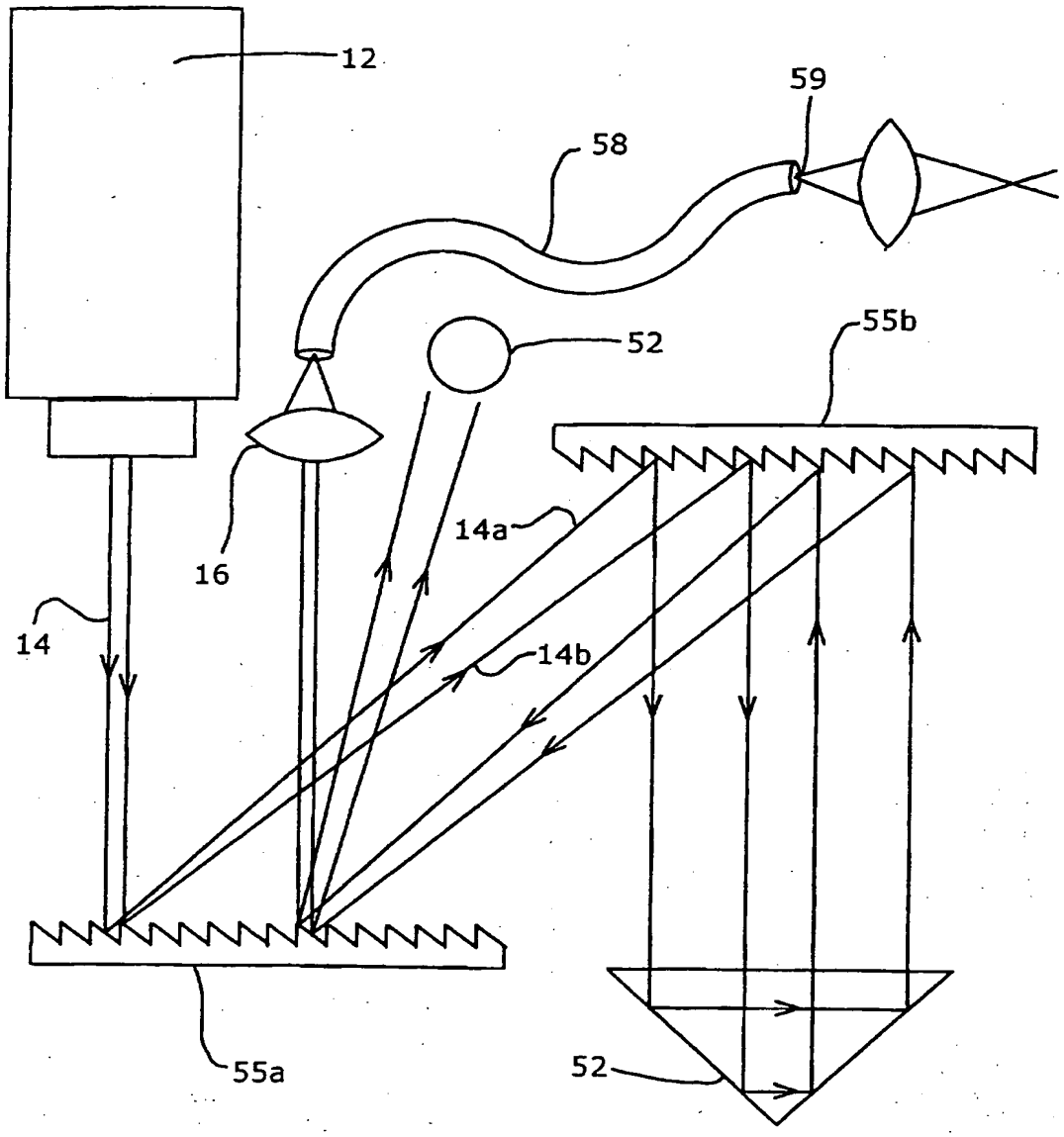

FIG. 1B

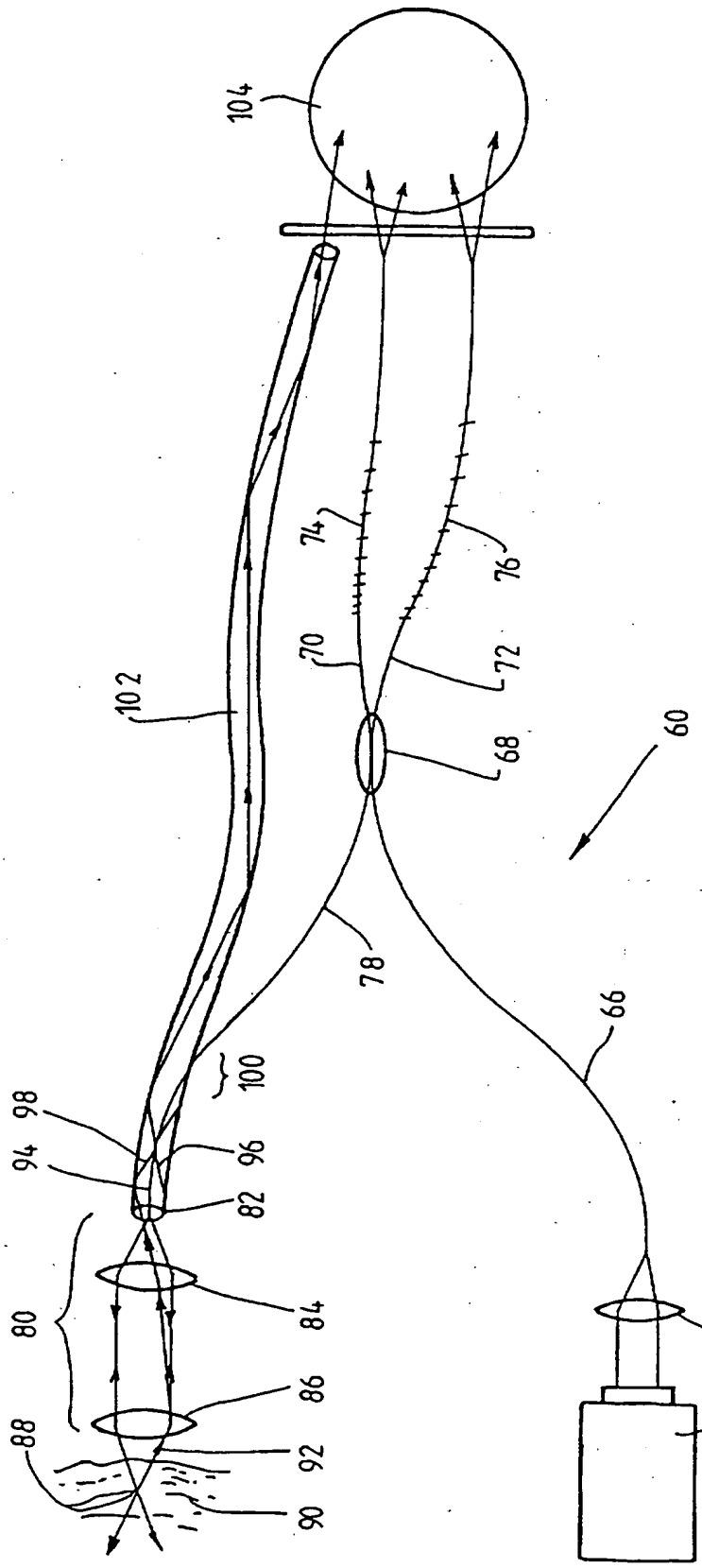

FIG. 2A.

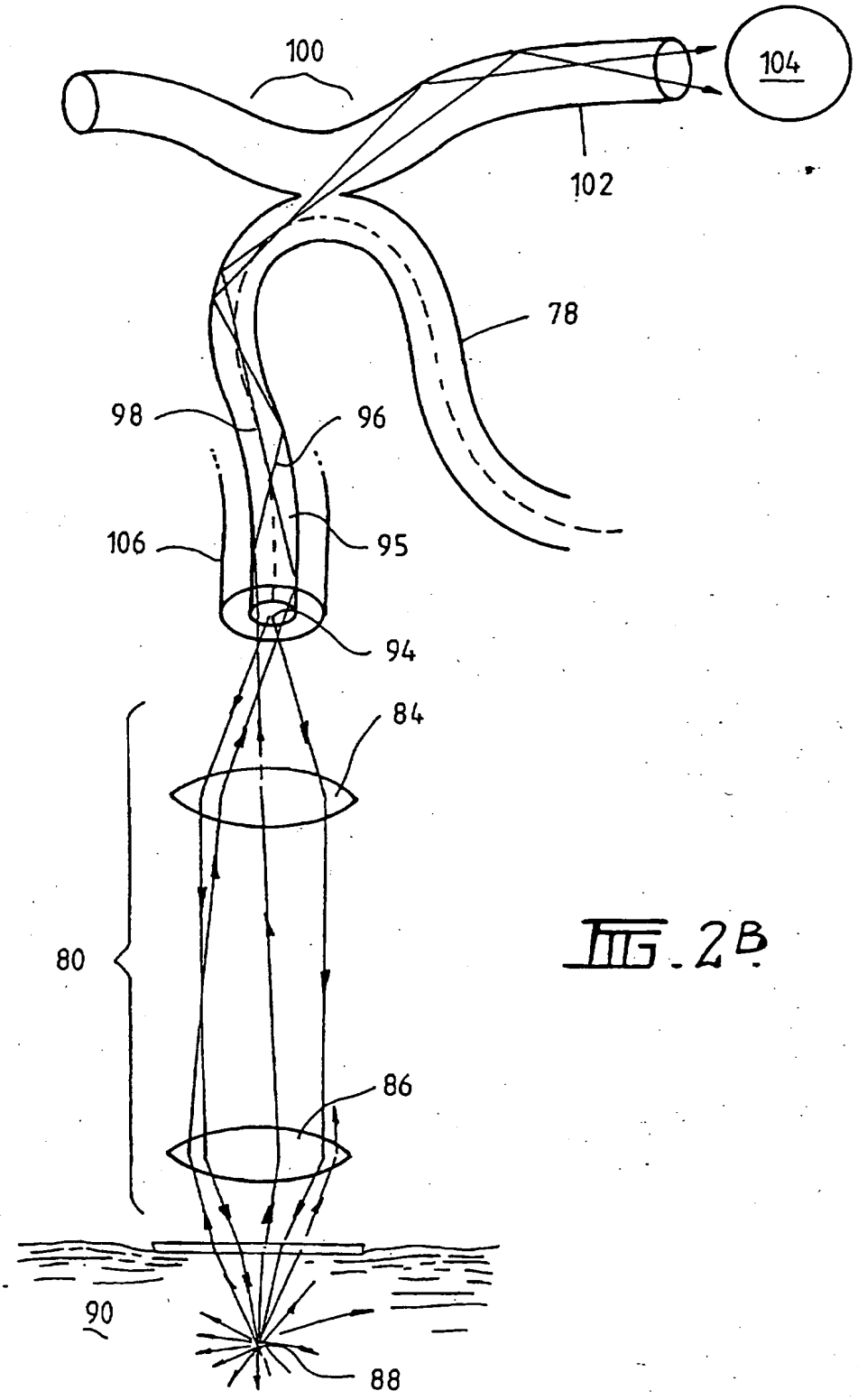

FIG. 2B

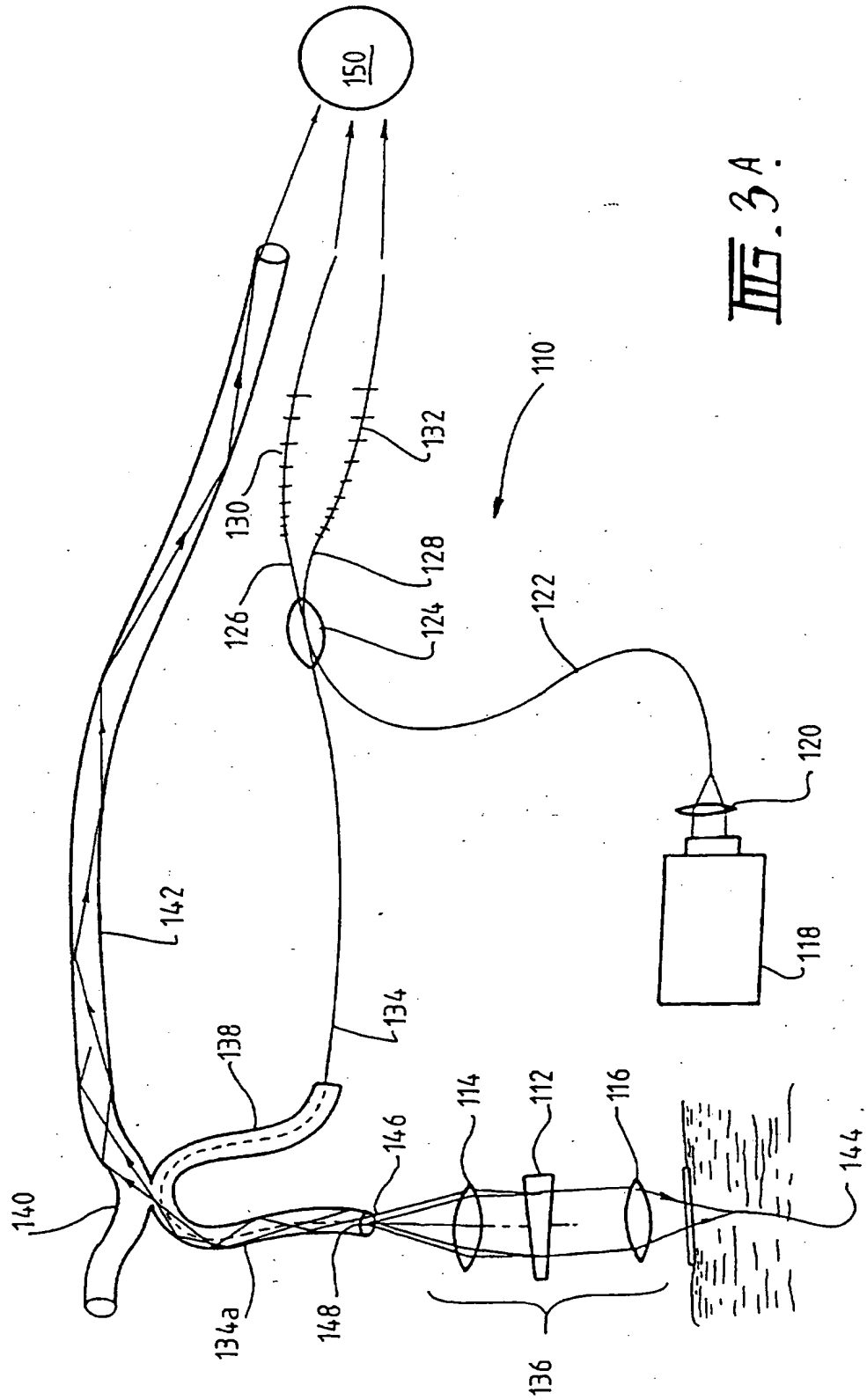
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FIG. 3A

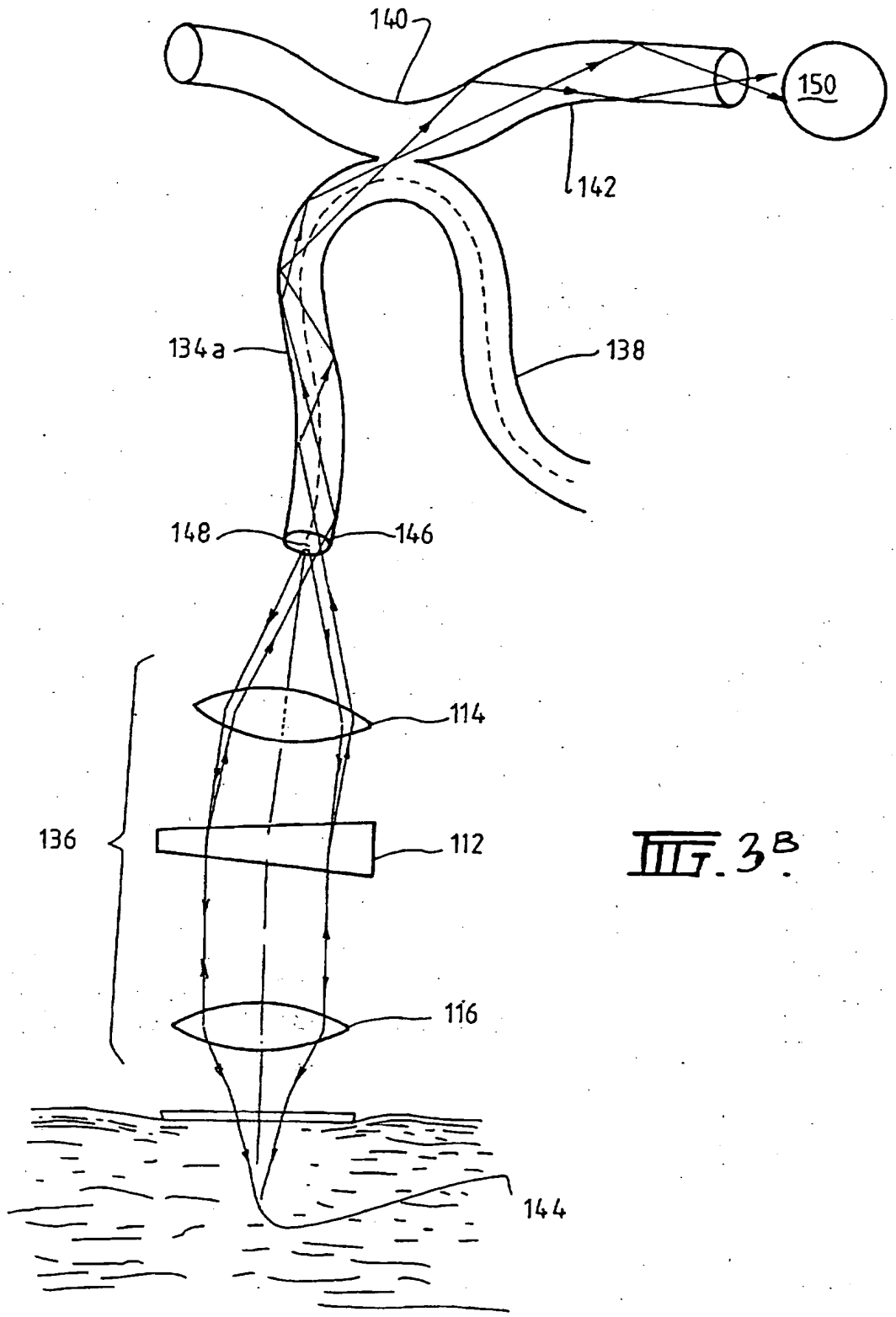
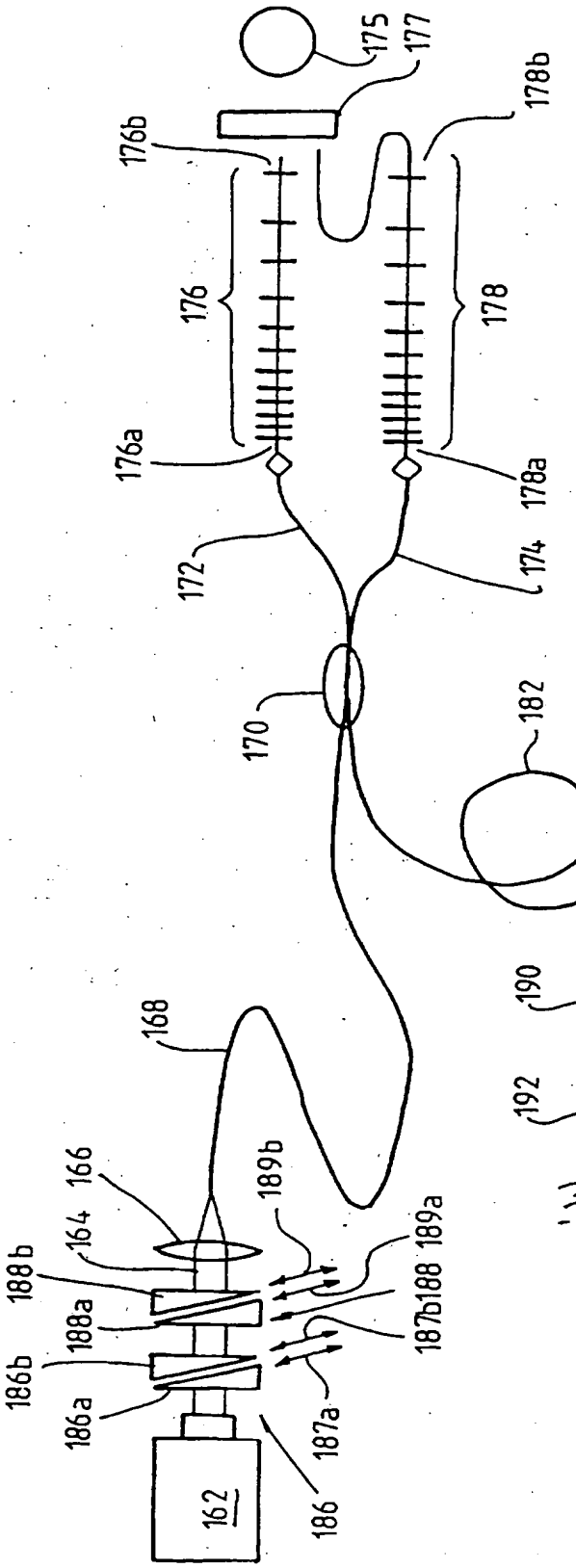


FIG. 3B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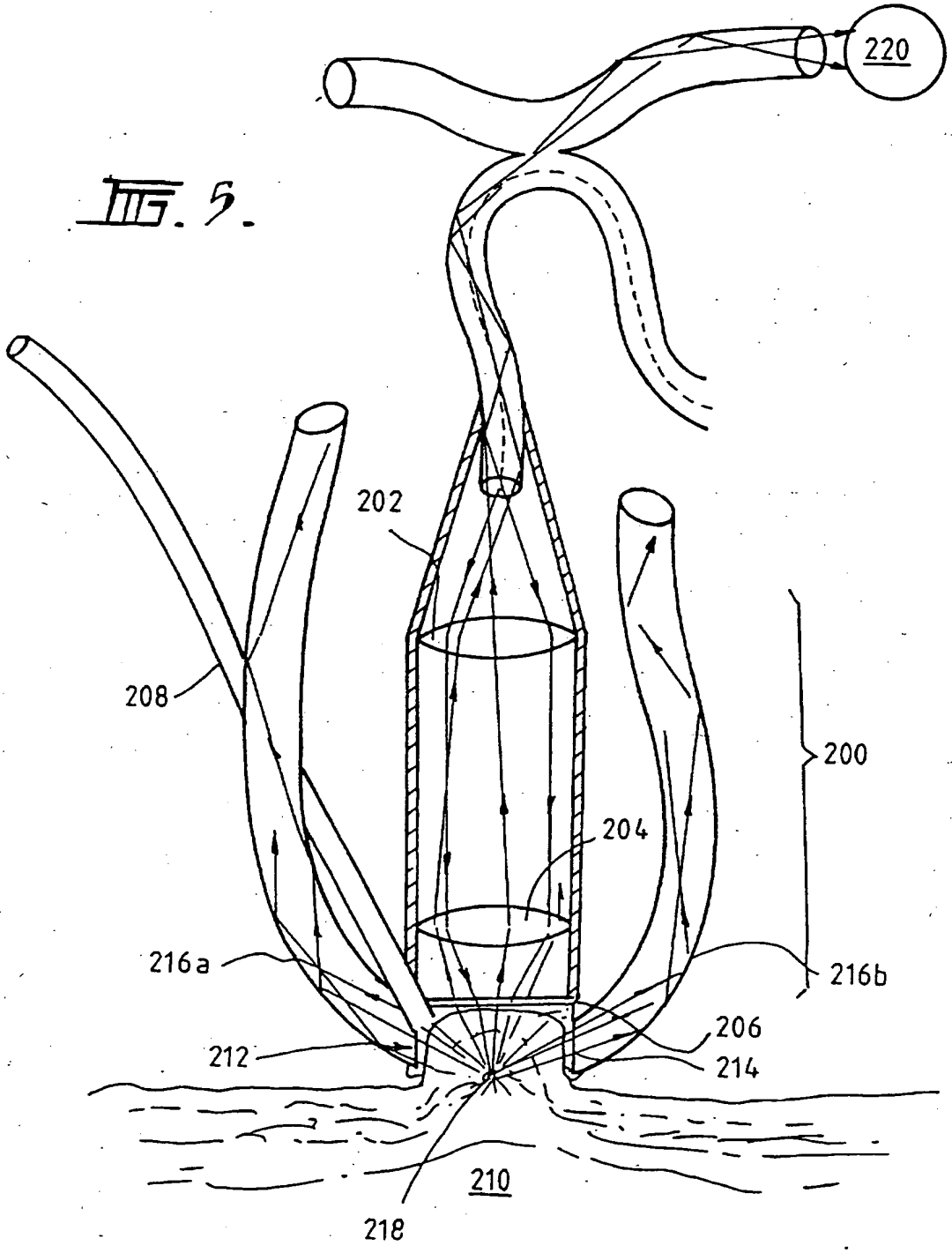
Фиг. 4.

FIG. 5.



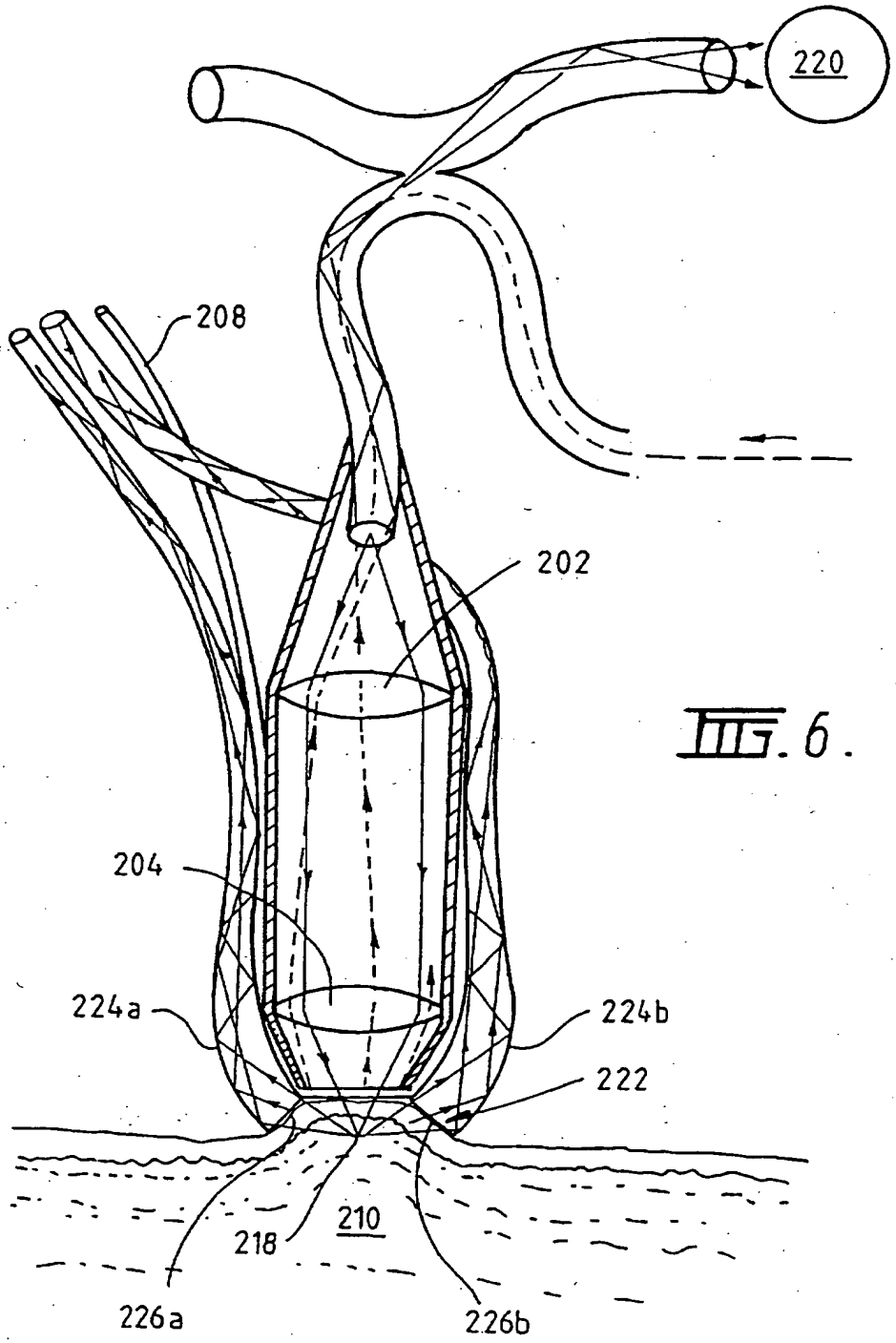


FIG. 6.

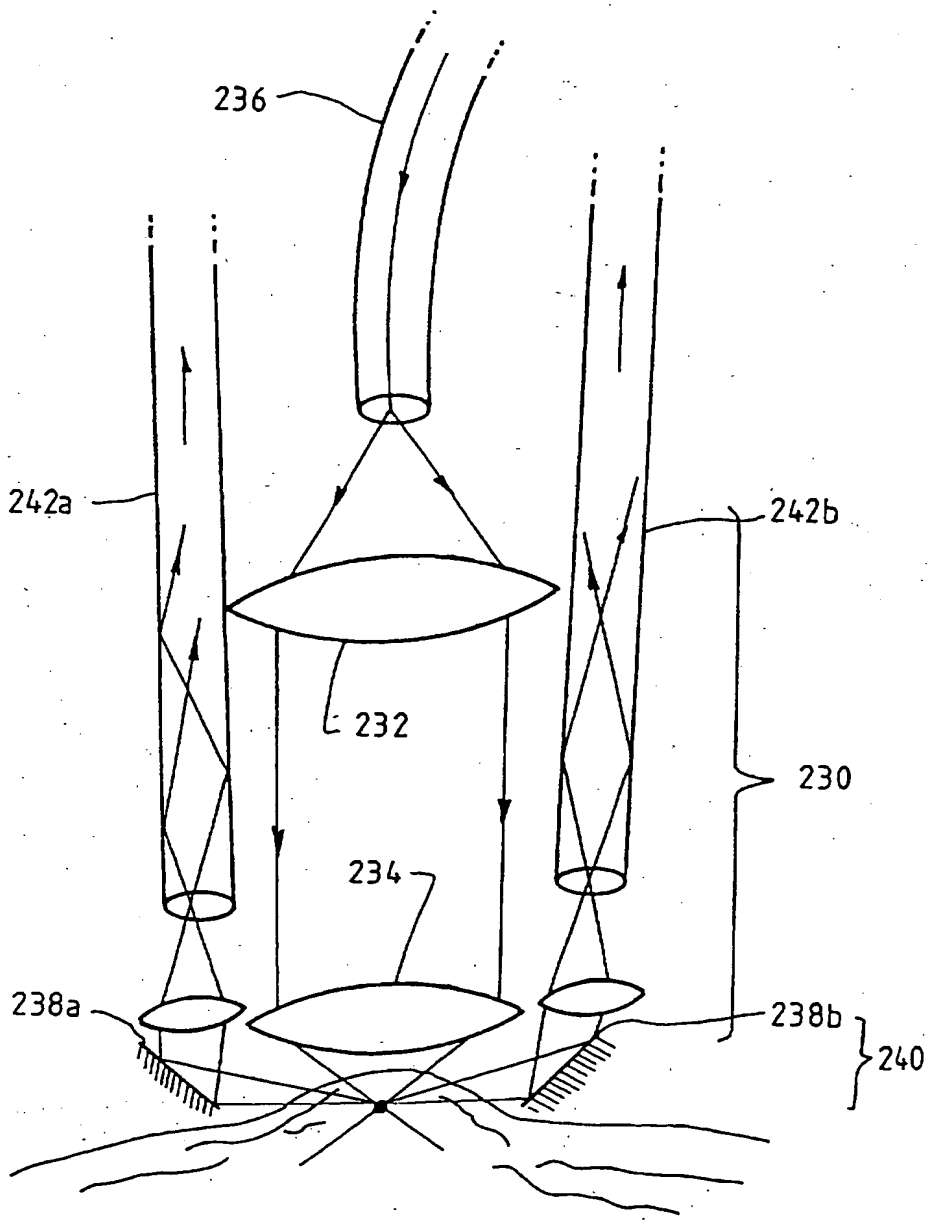
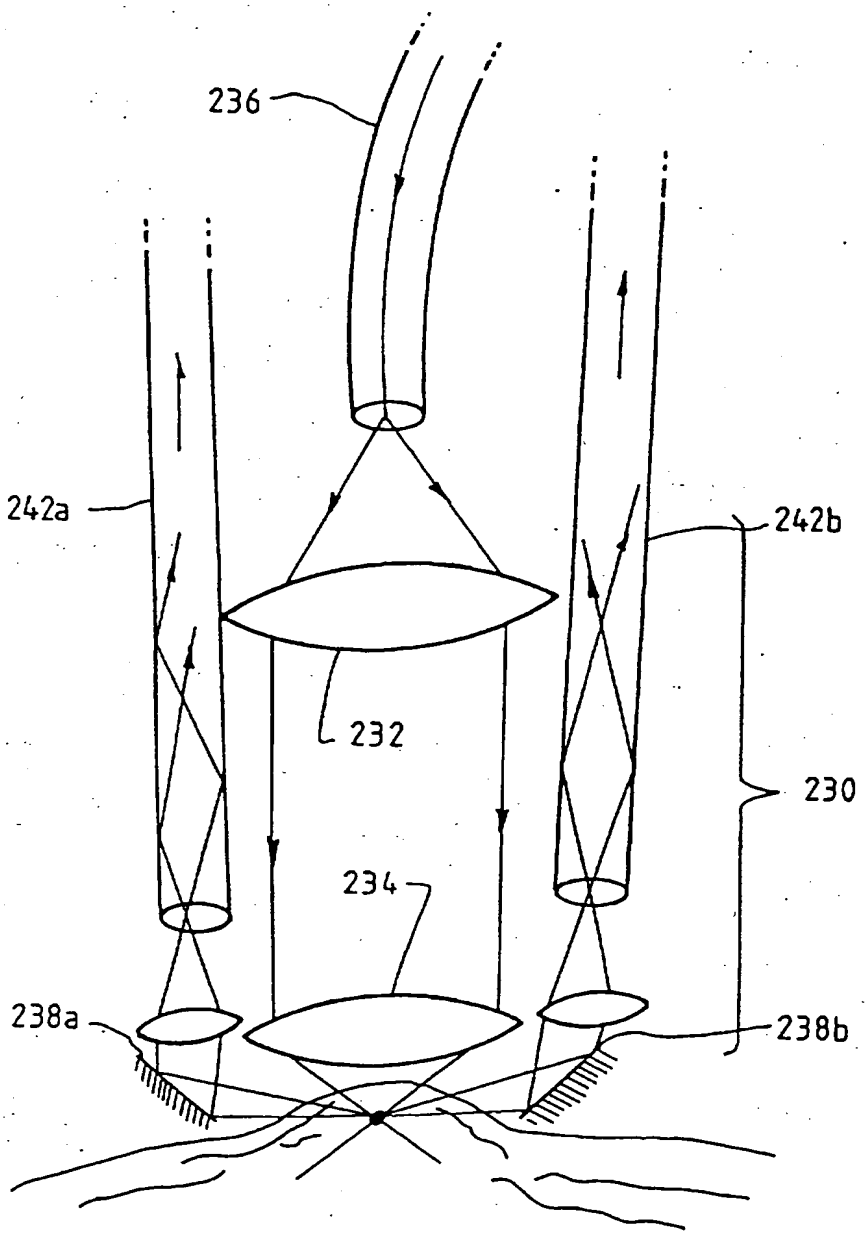


FIG. 7.



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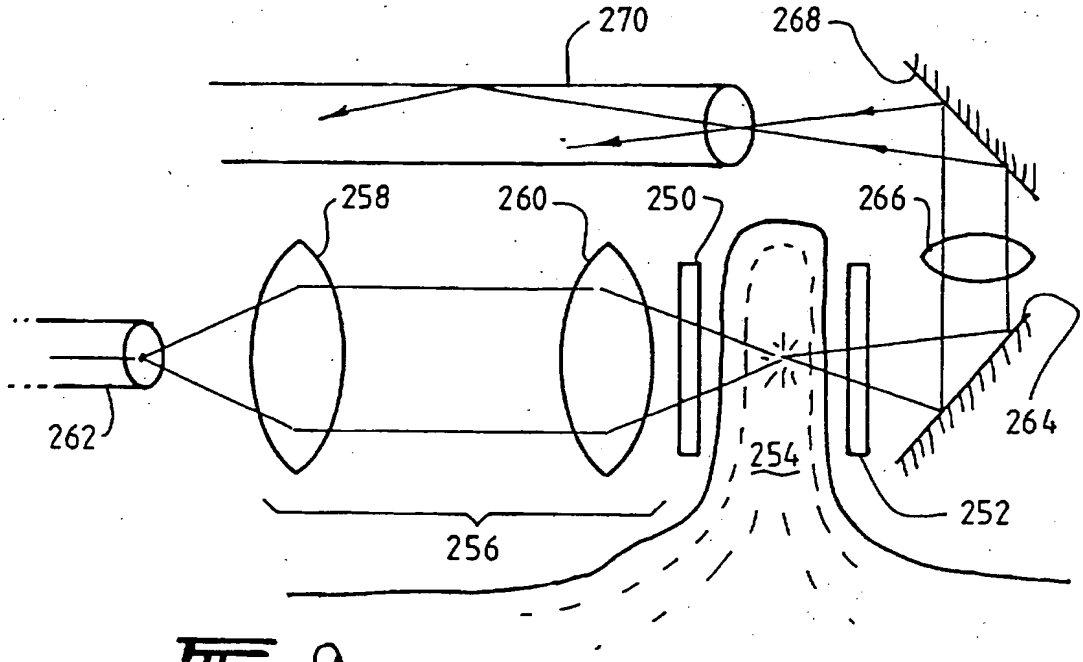
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FIG. 9.

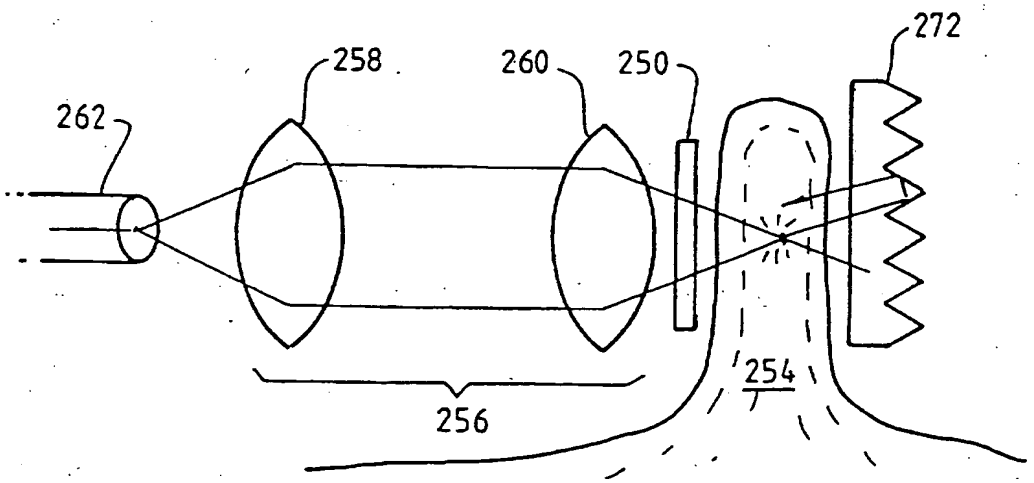
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FIG. 10.

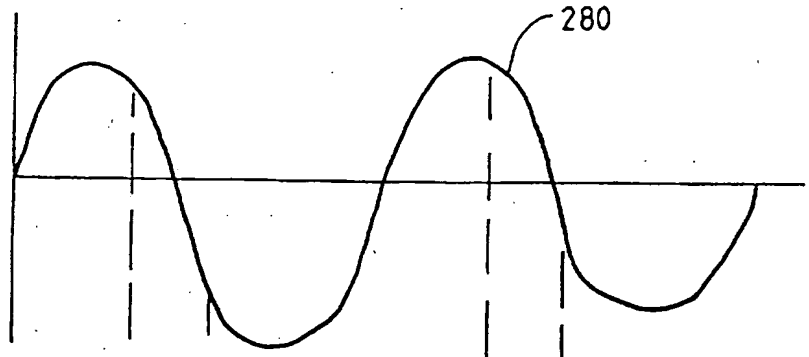

FIG. 11A.

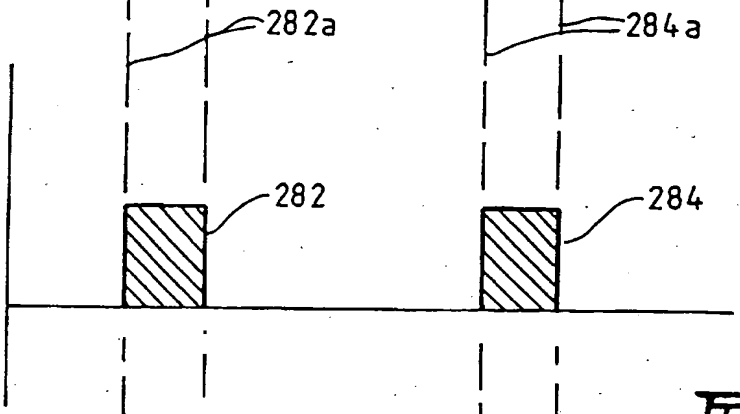

FIG. 11B

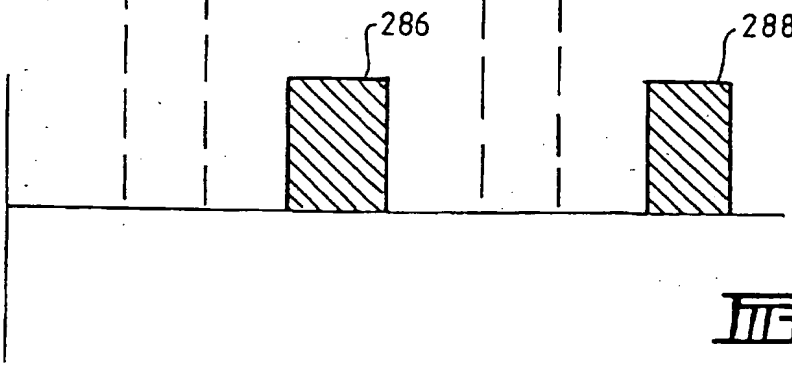

FIG. 11C

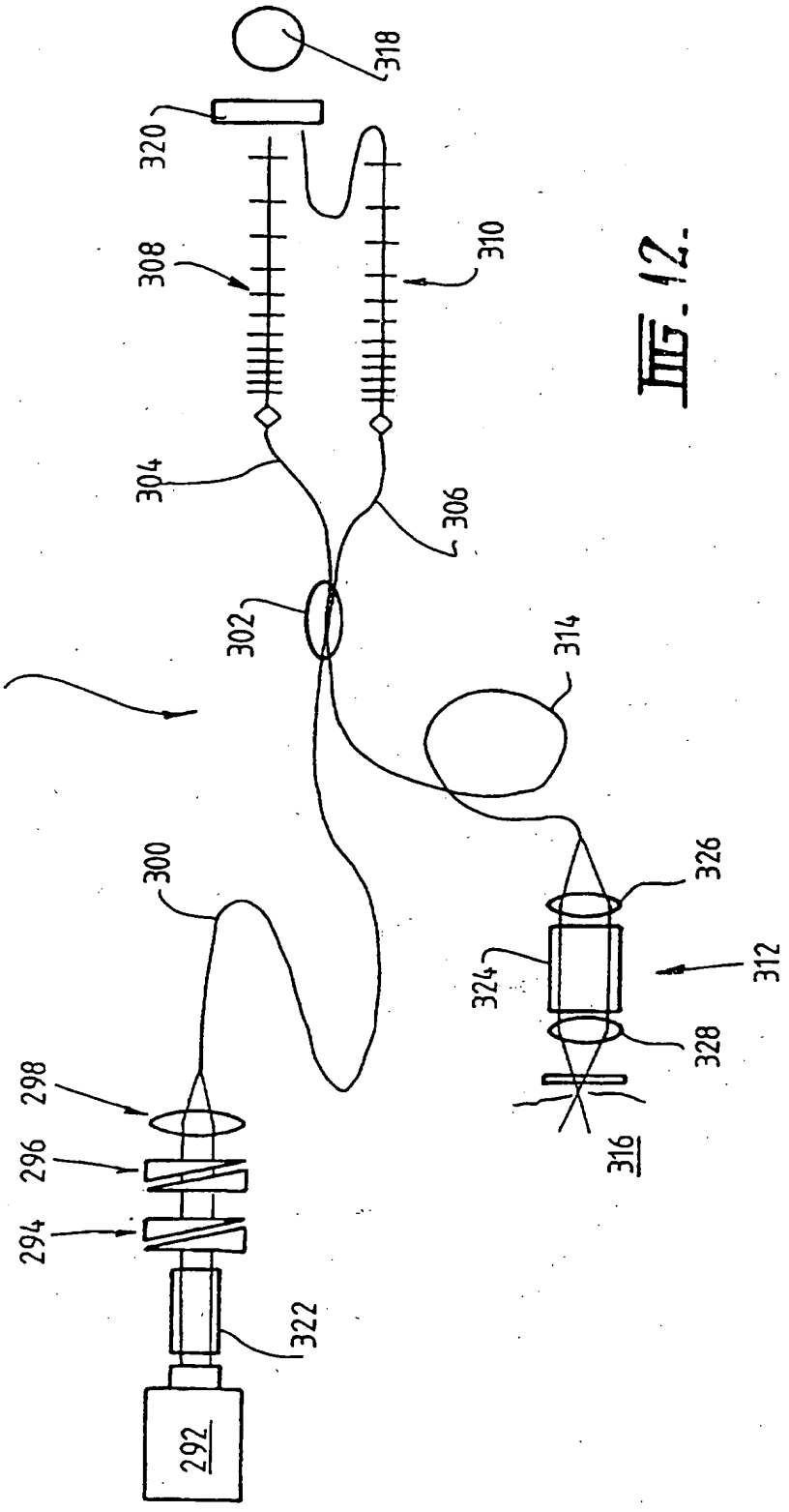
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FIG. 12.

TWO PHOTON ENDOSCOPE OR MICROSCOPE METHOD AND APPARATUS

FIELD OF THE INVENTION

The present invention relates to the field of endoscopy and microscopy, including endomicroscopy, and in particular to optical fibre endoscopy and microscopy (including endomicroscopy).

It should be understood that reference to microscopes hereinafter includes reference to endomicroscopes.

BACKGROUND OF THE INVENTION

Recently developed ultrashort pulse Ti:Sapphire lasers can produce extremely high peak optical power at the focus of a high NA lens and this allows non-linear optical effects to be generated with high efficiency. Two photon scanning spot excitation techniques have been found to have advantages for use in conventional bench microscopes.

Although the clinical use of two photon excitation might potentially offer the capability to obtain images from a greater depth below the surface of a tissue, reduced photobleaching and the ability to excite UV dyes and indicators without the use of unreliable UV lasers and special lenses, it has not been clear how two photon excitation could be usefully implemented in the area of clinical endomicroscopy. For example, a major drawback to the more widespread use of two photon scanning spot microscopy is the high cost of the short pulse laser.

More critically with regard to the present invention, the use of an optic fibre to deliver the laser light (as with earlier endomicroscope designs) stretches the pulse, which considerably reduces the peak intensity. This reduces the non-linear interaction efficiency and hence lowers the signal strength. Pulse broadening in a fibre in the linear domain is caused by chromatic temporal dispersion, the degree of which can be predicted from the Fourier transform of the pulse envelope or from Heisenberg's uncertainty

principle. When a pulse passes along the fibre the blue end of the Fourier transform travels more slowly than the red and a broad chirped pulse exits the fibre.

Thus, these two factors alone have appeared to rule out any consideration of the two photon endoscope as a clinical diagnostic instrument.

It is an object of the present invention, therefore, to provide an improved endoscope, microscope or endomicroscope by employing two photon excitation of a tissue.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides an endoscope or microscope including:

an optic fibre for transmitting a pulsed main beam along at least a portion of an optical path between a beam source and a tissue or other sample; and

a dispersing means;

whereby dispersion occurring in said optic fibre is reversed by dispersion produced by said dispersing means.

Preferably the dispersing means includes a compression means located optically after the optic fibre, for compressing the beam after the beam leaves the optic fibre, to reduce the peak power of the main beam within the optic fibre.

The dispersing means may be located optically before or after the optic fibre, and may itself include a further optic fibre.

Preferably the endoscope or microscope further includes a dispersion means located optically before the optic fibre, for dispersing said main beam before said main beam enters the optic fibre.

Each of the dispersion means and/or the compression means preferably comprises a block of glass, grating or prism.

Preferably each of the dispersion means and/or said compression comprises high dispersion glass.

Preferably the optic fibre has a large diameter core and hence low v number, to reduce areal power density in the core.

The optic fibre may have a pure silica core and fluoride doped cladding, to minimise non-linear optical effects within the fibre.

The present invention also provides a method of two photon endoscopy or microscopy including the steps of:

transmitting a pulsed main laser beam along an optic fibre along at least a portion of an optical path between a beam source and a tissue or other sample; and

dispersing said beam by means of dispersing means;

whereby dispersion occurring in said optic fibre is reversed by dispersion produced by said dispersing means.

In one particular aspect of the invention, there is provided an endoscope or microscope including:

a beam splitter means for splitting a pulsed main laser beam into first and second beams;

a first optic fibre for transmitting said main beam to said beam splitter means;

an antidispersive means for reducing the dispersion of said first and second beams;

an optical coupler means for recombining at least a portion of each of said first and second beams into a recombined beam; and

a second optic fibre for transmitting said recombined beam from said optical coupler means to an endoscope or microscope head;

wherein said main beam may be split by said beam splitter means into said first and second beams, whose dispersion may then be reduced by said antidispersive

means, and then recombined by said optical coupler means to form a recombined two photon beam for transmission along said second optic fibre to said head.

Thus, a pulsed laser beam can be converted into a two photon beam for use in an endoscope or microscope. The antidispersive means reduces the dispersion or compresses the first and second beams. It should be understood that the compression may occur or be completed outside the antidispersive means, and indeed in the endoscope or microscope head or even in a tissue sample.

Preferably said endoscope or microscope includes a laser source to provide said main beam.

Preferably said laser source is an ultrashort pulse laser and more preferably a femtosecond laser.

Preferably said laser source is a Titanium Sapphire laser or a $Cr:LiSrAlF_6$ laser.

Preferably said first optical fibre is single moded at the wavelength of said main beam.

Preferably said beam splitter means is an optical coupler, and more preferably a fused biconical taper coupler.

Preferably said beam splitter means divides said main beam approximately equally into said first and second beams.

Preferably said optical coupler means is an optical coupler, and more preferably a fused biconical taper coupler.

Preferably said beam splitter means and said optical coupler means are provided by a single optical coupler, and more preferably by a single fused biconical taper coupler.

Preferably said antidispersive means includes a reverse

chirped Bragg grating fibre whose more closely spaced gratings are located optically closer to said beam splitter means, and more preferably first and second reverse chirped Bragg grating fibres, one for receiving each of said first and second beams, each of whose more closely spaced gratings are located optically closer to said beam splitter means.

Thus, Bragg grating fibres may be used to compress the first and second beams. This also act as dichroic mirrors for selecting by dichroic beamsplitting the fluorescent light emitted by a tissue or other sample.

Preferably said endoscope or microscope further includes a large diameter optic fibre and a cladding mode coupler, wherein said cladding mode coupler couples said second optic fibre and said large diameter fibre so that large angle scattered light from said head will be substantially coupled into said large diameter fibre by said coupler for transmission to a photodetector.

In one preferred embodiment said endoscope or microscope includes a compression means located optically after said second optic fibre, for compressing said recombined beam after said recombined beam leaves said second optic fibre, whereby the peak power of said main beam within said first optic fibre and of said recombined beam in said second optic fibre is reduced.

Preferably the endoscope or microscope further includes a dispersion means located optically before the first optic fibre, for dispersing said main beam before said main beam enters said first optic fibre.

The dispersion means and/or the compression means preferably comprises a block of glass, grating or prism.

Preferably said dispersion means and/or said compression comprises high dispersion glass.

In one particular embodiment, said first optic fibre and said second optic fibre each has a large diameter core and hence low v number, to reduce areal power density in said cores.

In another particular embodiment, said first optic fibre and said second optic fibre have pure silica cores and fluoride doped cladding, to minimise non-linear optical effects within said fibres.

According to another aspect of the invention, there is provided an endoscope or microscope including:

a dispersing means for temporally dispersing a pulsed main laser beam spectroscopically; and

an optic fibre for transmitting said dispersed main beam to an endoscope or microscope head and reducing said dispersion by compressing said dispersed main beam.

Thus, rather than first allowing dispersion to occur in an optic fibre etc, then correcting that dispersion, the dispersion can be initially introduced in an opposite sense and then be removed in the transmission by the fibre of the beam to the sample.

The optic fibre may substantially reverse the dispersion introduced by the dispersing means. Alternatively, the endoscope or microscope head may include a compression means located optically after the optic fibre, for furthering or completing the compression of the dispersed main beam, to reduce the peak power of the main beam within the optic fibre.

Preferably the dispersing means is also a beam splitter means for selecting the fluorescent light emitted by a tissue or other sample.

Preferably the dispersing means includes a pair of diffraction or refraction elements, and more preferably a

pair of blazed gratings and a prism.

Also according to the invention, there is provided an endoscope or microscope with a head, said endoscope or microscope including:

a suction or partial vacuum source located near said head;

a light collecting means for collecting light from a sample; and

a sample receiving means coupled to said suction source for receiving said sample when said sample is urged thereto by said suction source;

wherein said suction source is used to urge said sample towards said receiving means, thereby deforming said sample so that said light collecting means can collect some of light emitted by said sample away from said head.

Preferably said sample receiving means is cup shaped or alternatively frustum shaped.

Preferably said light collecting means is in use separated from said sample by said sample receiving means, and said sample receiving means is substantially transparent to said light between said sample and said light collecting means.

The light collecting means may comprise an optic fibre.

Alternatively said light collecting means may comprise a mirror. The endoscope or microscope may include a lens for focussing light from said mirror.

Preferably said light collecting means is one of a plurality of light collecting means.

The invention also provides a method of two photon endoscopy or microscopy including the steps of:

1) transmitting a pulsed main laser beam along an optic fibre;

2) splitting said main beam into first and second

beams;

3) compressing said first and second beams;

4) combining at least a portion of said first and second beams;

5) transmitting said first and second beams along an optic fibre to an endoscope or microscope head.

In one specific embodiment, the present invention provides an endoscope or microscope system including:

an endoscope or microscope, including a photodetector;

an illumination means for providing ambient illumination;

switching means for switching said illumination means on and off; and

synchronization means for synchronizing said switching means with acquisition periods of said endoscope or microscope;

wherein said synchronization means is operable to control said switching means to switch said illumination means off during said acquisition periods and on between consecutive acquisition periods.

Preferably said synchronisation means is operable to control said switching means to switch said illumination means such that said illumination means is on during the flyback period of said endoscope or said microscope.

The photodetector may be operable to be switched off when said illumination means is on.

Preferably said photodetector has a power supply operable to be switched off when said illumination means is on.

Preferably the photodetector includes a photo multiplier tube and said power supply is an EHT power supply.

In another specific embodiment, the present invention provides an endoscope or microscope system including:

an endoscope or microscope, including a photodetector;

an illumination means for providing ambient illumination; and

filter means for reducing the detection of said ambient illumination by said photodetector.

Preferably the illumination means emits light preferentially blocked by said filter means.

Alternatively, the filter means is a first filter means, and the system includes a second filter means for absorbing light from said illumination means that is preferentially passed by said first filter means and transmitting light from said illumination means that is preferentially blocked by said filter means.

Preferably the system includes switching means for switching said illumination means on and off, and synchronization means for synchronizing said switching means with acquisition periods of said endoscope or microscope, wherein said synchronization means is operable to control said switching means to switch said illumination means off during said acquisition periods and on between consecutive acquisition periods.

Preferably said system includes a shutter for occluding said photodetector, wherein said synchronization means is operable to control said shutter means to occlude said photodetector when said illumination means is on, and expose said photodetector during said acquisition periods.

Thus, the light detecting means (generally a photo-multiplier tube) will be protected from exposure to the ambient light during non-acquisition periods, to reduce noise levels.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the present invention may be more clearly

ascertained, preferred embodiments of the present invention will now be described, by way of example, with reference to the accompanying drawings in which:

Figure 1A is a schematic view of a two photon optic fibre endomicroscope according to a preferred embodiment of the present invention;

Figure 1B is a schematic view of a two photon optic fibre endomicroscope according to a alternative preferred embodiment of the present invention;

Figure 2A is a schematic view of a two photon optic fibre endomicroscope according to another preferred embodiment of the present invention;

Figure 2B is a more detailed view including the head of the endomicroscope of figure 2A;

Figure 3A is a schematic view of a two photon optic fibre endomicroscope according to yet another preferred embodiment of the present invention;

Figure 3B is an enlarged view of a detail of figure 3A;

Figure 4 is a view of a portion of an endomicroscope according to still another preferred embodiment of the present invention;

Figure 5 is a view of the endomicroscope head arrangement for use with the endomicroscope of figure 2 or 3;

Figure 6 is a view of another endomicroscope head arrangement for use with the endomicroscope of figure 2 or 3;

Figure 7 is a view of an endomicroscope head arrangement for use with an endomicroscope according to the present invention;

Figure 8 is a view of another endomicroscope head arrangement for use with an endomicroscope according to the present invention;

Figure 9 is a view of yet another endomicroscope head arrangement for use with an endomicroscope according to the present invention;

Figure 10 is a view of still another endomicroscope head arrangement for use with an endomicroscope according

to the present invention, being a variation of the arrangement of figure 9;

Figures 11A, 11B and 11C are schematic graphs illustrating a method of employing an endomicroscope according to the present invention; and

Figure 12 is a view of a portion of an endomicroscope according to yet a further preferred embodiment of the present invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A two photon optic fibre endomicroscope according to a preferred embodiment of the present invention is shown schematically at 10 in figure 1A. The endomicroscope 10 includes a laser source in the form of femtosecond laser 12 for producing a rapidly pulsed beam 14, a lens 16 for focussing beam 14, and an optic fibre 20 with a core 18. The lens 16 is used to focus beam 14 into core 18. Optic fibre 20 is single moded at the wavelength of the laser 12.

Each pulse of beam 14 travels along the fibre 20 to a beam splitter in the form of fused biconical taper coupler 22. The coupler 22 divides the main beam 14 roughly equally into two beams, so that pulses of each of these beams are transmitted from coupler 22 along the two output legs of the coupler, optic fibres 24 and 26 respectively. Optic fibres 24 and 26 include antidispersive means in the form of reverse chirped Bragg grating fibres 28 and 30 respectively, oriented with the shorter spaced grating elements 28a and 30a nearer the coupler 22 than the longer spaced grating elements 28b and 30b. Thus, the pulses of each of these beams encounter gratings 28 and 30 respectively, after leaving coupler 22.

The pulses are reflected back from gratings 28 and 30 towards the coupler 22 with the blue, shorter wavelength components of each pulse ahead of the red, longer wavelength components of the pulse. The pulses arrive at the coupler 22 close together but not exactly in phase. There is no attempt to match exactly the fibre distances

from the Bragg gratings 28 and 30 to the coupler 22. Each pulse is again divided by the coupler 22, so that two (a component of each pulse reflected from gratings 28 and 30) are directed back along fibre 20 towards the laser 12 and two pulses (also a component of each pulse reflected from gratings 28 and 30) is directed along fibre 32. The pulses in fibre 32 gradually bunch up as the red components catches up with the blue. The pulses leaving fibre tip 34 are thus almost recombined back as a pulse (allowing for compensation of final chromatic differential delays in the lenses 36 and 38 of the endomicroscope head 40).

The pulses directed along fibre 32 are focussed by lenses 36 and 38 to a Gaussian waist 42 within the tissue 44 to be examined. Scanning of the Gaussian waist 42 within the tissue may be achieved by mechanisms 46 actuating motion of the fibre tip 34 in a raster or by mirror scanning or other desired scan pattern-generation.

Fluorescence generated at the Gaussian waist 42 by two photon excitation of fluorophores within the tissue 44 travels back through the lenses 38 and 36 and is focussed back into the core of the fibre 32. The fluorescent light then travels back along the fibre 32 to the coupler 22. It is then split by the coupler 22 and travels along fibres 24 and 26. The fluorescent light, being of a much shorter wavelength than main beam 14, passes through the Bragg mirrors 28 and 30 (which thus act as dichroic beamsplitters) to the ends 48 and 50 of the fibres 24 and 26, from which it passes to the photomultiplier tube 52 after passing through BG39 spectral filter 54. Conventional acrylate coating fibres and communications couplers are quite suitable for this system.

A two photon optic fibre endomicroscope according to an alternative preferred embodiment of the present invention is shown schematically in figure 1B, in which like reference numerals are used to designate like components from figure 1A. Unlike the embodiment of figure 1A,

however, a dispersing means comprising first and second blazed gratings 55a and 55b and 90° retro-reflector prism 56 is disposed optically between laser 12 and lens 16. The location of maxima in the light diffracted by first grating 55a is wavelength dependent, so the beam 14 is effectively spread spatially into its component frequencies: this is depicted schematically in terms of shorter wavelength light 14a and longer wavelength light 14b. The beam is then reflected by second grating 55b towards prism 56 (or, alternatively, a mirror) which returns the light, via second grating 55b to first grating 55a. The combination of gratings 55a and 55b and prism 56 introduces a path difference between shorter and longer wavelength components 14a and 14b of beam 14, and an offset at first grating 55a, so that the beam is then directed towards lens 16. Lens 16 focusses the components of the now dispersed beam pulses into optic fibre 58, with shorter wavelength components leading longer wavelength components. As these components travel along fibre 58, the longer wavelength components gradually catch up with the shorter wavelength components. The pulses leaving fibre tip 59 are thus almost recombined back as a pulse (allowing for compensation of final chromatic differential delays in the subsequent lenses of the endomicroscope head, as discussed above).

Fluorescence generated in the sample is, as in the embodiment of figure 1A, focussed back into the core of the fibre 58, travels along fibre 58 to lens 16, and is reflected from first grating 55a towards to the photomultiplier tube 52 after passing through a BG39 spectral filter (not shown). The return fluorescence is two photon induced, so it has a different range of wavelengths from that of the beam 14. Consequently, it is diffracted at a different angle from pulsed beam 14 and may be conveniently detected by photomultiplier tube 52 without interfering with the optical path of components 14a and 14b of beam 14.

An endomicroscope according to a further preferred

embodiment of the present invention is shown at 60 in figure 2A. Endomicroscope 60 is similar to endomicroscope 10 of figure 1A, and includes laser source 62, lens 64 optic fibre 66, coupler 68 with two output legs (fibres 70 and 72) each of which includes a reverse chirped Bragg grating fibre 74 and 76, except that the last section of glass clad optic fibre 78 leading from coupler 68 to the endomicroscope head 80 is jacketed with a low refractive index material (not shown in this figure) such as low RI silicone rubber, which allows the transmission of cladding modes. Pulsed laser light emerging from fibre tip 82 is focussed by lenses 84 and 86 into a Gaussian waist 88 within the tissue 90.

Owing to 'snake light' 92 (low angle forward scattering of the 2ω excited fluorescent light) emitted from the Gaussian waist region 88, much of the fluorescence misses the core 94 of fibre 78 on the return, but enters the glass cladding of fibre 78. These snake light rays are bound by the low RI silicone rubber jacket and propagate as cladding modes 96 and 98 until they encounter cladding mode coupler 100.

At this point the endomicroscope 60 includes - coupled by cladding mode coupler 100 to fibre 78 - a large diameter fibre 102. Thus, the cladding modes 96 and 98 are largely coupled out into the larger diameter fibre 102 and proceed along this fibre 102 to the photomultiplier tube 104 which generates a signal for a suitable image acquisition system (not shown).

Figure 2B is a more detailed view of the endomicroscope head 80 of figure 2A. Two photon generated fluorescence 92 is emitted at the laser focal point 88 within the tissue 90. The shorter wavelength two photon fluorescence is more subject to small angle forward scattering ('snake scattered light'), and consequently - as described above - the return light passing back through the lenses 86 and 84 of the endomicroscope head 80 will not re-focus to the core 94 of the fibre 78. Instead, the snake scattered light enters

the glass cladding 95 and is trapped by the low RI clear silicone rubber jacket 106. A cladding mode coupler 100 takes most of this light into the larger diameter fibre and carries it to the photomultiplier tube 104.

Figure 3A is a view of yet another preferred embodiment of an endomicroscope 110 according to the present invention. In this embodiment, the endomicroscope 110 includes a very shallow angle prism 112, interposed in the optical train between the lenses 114 and 116.

In other respects, endomicroscope 110 is similar to endomicroscope 60 of figures 2A and 2B. Thus, endomicroscope 110 includes laser source 118, lens 120, optic fibre 122, coupler 124 with two output legs (fibres 126 and 128) each of which includes a reverse chirped Bragg grating fibre 130 and 132, optic fibre 134 from coupler 124 to endomicroscope head 136. Optic fibre 134 has glass cladding 138 (shown for simplicity only in the region of cladding mode coupler 140 and endomicroscope head 136).

Endomicroscope 110 also includes the cladding mode coupler 140 mentioned above, and large diameter fibre 142.

Prism 112 does not have sufficient dispersion to spread the excitation beam at the Gaussian waist region 144 by any appreciable amount, but it will deviate the shorter fluorescence wavelengths by an amount sufficient that they are returned laterally displaced to the side 146 of the excitation wavelength emitting core 148.

This ensures that most or all the 2ω excited fluorescence are returned as cladding modes in the light delivery fibre 134a. These modes are coupled out into large diameter fibre 142 by cladding mode coupler 140 and are carried by fibre 142 to the photomultiplier tube 150.

This would allow a simpler optical return path for the fluorescence being entirely via the cladding mode coupler

140 fibre.

Although not illustrated, a similar effect could be achieved without the need for prism 112 if lenses 114 and 116 have a slight amount of chromatic aberration.

Figure 3B is an enlarged view of the endomicroscope head 136 of figure 3A, with fibre 138, large diameter fibre 142 and cladding mode coupler 140. In this figure, the lateral displacement of the shorter fluorescence wavelengths rays to the side 146 of the core 148 of fibre 138 is more clearly visible.

Figure 4 is a schematic view of an endomicroscope 160 according to a further preferred embodiment of the present invention. Endomicroscope 160 includes a laser source in the form of Ti:Sapphire laser 162 for providing laser beam 164, focussing lens 166 and optic fibre 168. Optic fibre 168 directs beam 164 to fused biconical taper coupler 170, which splits beam 164 into two approximately equal beams directed along fibres 172 and 174 respectively. As in other preferred embodiments described above, fibres 172 and 174 each include a reverse chirped Bragg grating fibre 176 and 178 respectively. These Bragg gratings 176 and 178 are oriented with the shorter spaced grating elements 176a and 178a nearer the coupler 170 than the longer spaced grating elements 176b and 178b.

An endomicroscope head 180 is provided, optically connected to coupler 170 by optic fibre 182. Figure 4 illustrates the head 180 located for examination of a tissue sample 184.

Fluorescent light is detected by photomultiplier tube 175 after passing through BG39 spectral filter 177.

Endomicroscope 160 also includes two sets of prisms 186 and 188, located optically between laser 162 and focussing lens 166.

Each prism set 186 and 188 comprises a pair of closely positioned optical wedge prisms 186a,b and 188a,b respectively.

The prisms 186a,b and 188a,b are moveable along tracks 187a,b and 189a,b respectively, either by an actuator motor or motors (not shown) or manually, so that the optical path length traversed by the beam within the endomicroscope 160 can be adjusted. The movement of the prisms is parallel to the oblique adjacent faces of each set of prisms 186 or 188. In the illustrated form of this embodiment, all four prisms are moveable. However, it may be preferred for simplicity that prisms 186a and 188a or 186b and 188b be moveable.

Prisms 186a and 186b are of fused silica or germanium doped silica (or other material having relatively linear dispersion) and can be adjusted to compensate for overall fibre length variability between systems.

Prism 188a and 188b are made of flint glass or other material with highly curved dispersion curves and can be moved in and out to adjust the chirp linearity. Both sets 186 and 188 of prisms are intended to be used to compensate variation in glass optical path length and also for variations which might be introduced when the final lenses 190 and 192 of head 180 are changed.

Prism sets 186 and 188 then allow the tuning (by means of Bragg grating fibres 176 and 178) of the overcompensated system. Further, the prism sets 186 and 188 permit adjustments to be made to the optical path length distance, in order to keep variability between the fibre systems within the optical path length distance.

Figure 5 is a view of the head region of an endomicroscope according to another preferred embodiment of the present invention, in use. In this embodiment, the endomicroscope

uses suction to draw tissue to be examined into a cup provided adjacent the front optical surface of the endomicroscope head.

Thus, the endomicroscope of this embodiment includes a head 200 including lenses 202 and 204 and front optical plate 206. There is also provided a suction tube 208 (attached to any suitable vacuum or reduced pressure source not shown) to suck tissue 210 to be examined into a cup 212 formed by transparent wall 214 and front optical plate 206 of the endomicroscope head 200. Tube 208 attached to cup 212 so that the suction provided by the tube 208 will draw the tissue 210 into the cup and against plate 206.

One or more optical fibres 216a,b are arranged around the wall 214 of the cup 212 with their ends pointing towards the area being examined within cup 212. These fibres 216 collect that fluorescent light from focal point 218 that does not enter the lens 204, and hence would not otherwise contribute to the signal. Thus, at least some of such light directed against wall 214 will be received by optic fibres 216 and convey to a photomultiplier tube 220.

Figure 6 shows a similar arrangement to that of figure 5, differing only in that cup 222 is frustum shaped, and optic fibres 224a,b have tapered 'solar concentrator' fibre tips 226a,b respectively to reduce the diameter of the bundle of fibres leading to the photodetector 220.

Figure 7 is a schematic view of an arrangement according to the present invention, in which additional fluorescent light is collected not by placing optic fibres adjacent the laser focal point in the tissue being examined, but by employing one or more mirrors.

Thus, endomicroscope head 230, which includes lenses 232 and 234, and is attached to optic fibre 236, also includes mirrors 238a,b located at the forward end 240 of head 230. Fluorescent light that - in the embodiment of figures 5 and

6 - would be captured by fibres 216 or 224 are instead reflected by mirrors 238 into fibres 242a,b, extending parallel to the main axis of the endomicroscope head 230. Lenses 244a,b are located between mirrors 238a,b and fibres 242a,b respectively to focus light into the fibres 242a,b and hence capture as much light as possible.

These lenses 244 are optional, however, and - as shown in figure 8 - it may be desirable (for the sake of compactness of lightness of the endomicroscope head or otherwise) to exclude them. Such an arrangement is shown in figure 8, in which reference numerals correspond to those of figure 7.

The arrangements of figures 7 and 8 avoid having curved fibres leading from the area surrounding the tip, as in the embodiments of figures 5 and 6.

These arrangements may also, however, employ a suction tube and cup (such as those shown in figures 5 and 6), with mirrors 238 located adjacent and outside the cup.

It should be noted that in any of the embodiments that use suction to draw tissue into a cup, the collection of light may be from a solid angle which exceeds 2π steradians, a much higher collection efficiency than is possible for a conventional single photon LSCM endoscope.

Vacuum or suction 'cupping' may tend to engorge the area to be examined with blood by artificially dilating the microvasculature. This has the potential to reduce the signal level from the fluorescence as haemoglobin has strong absorption bands in the blue green and yellow. A way of overcoming this would involve the use of a thread around the rim of the 'cup'. Contracting this ring of thread would compress a 'button' of tissue and would blanch it by squeezing the blood out while still maintaining the contact of the tissue with the front optical surface of the endomicroscope.

An alternative way of achieving one or more of these advantages (of greater solid angle of light gathering, and the exclusion of extra blood) involve the pinching and holding of a flap of sample tissue against the first optical surface of the endomicroscope. In this case optical return might also be obtained from the back of the flap. Examples of these embodiments are illustrated in figures 9 and 10.

In one embodiment of the invention (illustrated in figure 9), the endomicroscope is provided with a pinching means in the form of transparent plates 250 and 252 for pinching a sample of tissue 254. Plate 250 also serves as the forward optical element of endomicroscope head 256, which also includes lenses 258 and 260. The head 256 is attached to optic fibre 262, from which a pulsed laser beam is delivered.

This arrangement also includes a first mirror 264 located behind plate 252, lens 266 and second mirror 268. Fluorescent light scattered back out of tissue 254 through plate 252 is reflected by mirror 264, focussed by lens 266, and then reflected by second mirror 268 into optic fibre 270, thereby increasing the total detected solid angle.

Plates 250 and 252 - if pressed harder against tissue 254 - will blanch tissue 254 as described above, thereby reducing the excess accumulation of blood in the tissue 254.

Clearly mirrors 264 and 268 could be omitted from this arrangement, and light focused directly into fibre 270 by lens 266 by orienting lens 266 at 90° to that shown in figure 9, and locating fibre 270 directly behind plate 252. However, the arrangement shown is preferred owing to its greater compactness.

Alternatively, mirrors 264 and 268, lens 266 and fibre 270 could be eliminated if plate 252 is replaced with a retro-

reflector plate, as illustrated in figure 10.

In this arrangement, the tissue 254 is again clamped, but between plate 250 and retro-reflector plate 272. A retro-reflector plate has the property that light is reflected back in a direction parallel to that of incidence, though spatially displaced. Thus, light scattered back out of tissue 254 towards plate 272 will be reflected back through tissue 254 and collected by any suitable method, such as those described above with reference to any of figures 1, 2A 3A or 4.

However, owing to the wide angular range in the optical collection, the corner cubes 274 of retro-reflector plate 272 cannot rely on total internal reflection, and so the retro-reflector plate 272 is metallised.

A method to facilitate use of the two photon endomicroscope in situations where a high level of illumination is required during clinical examination is now described with reference to figures 11A, 11B and 11C.

In single photon confocal microscopy and endomicroscopy the return light is spatially filtered by the pinhole which effectively eliminates interference from room light. In the two photon endomicroscope it would be desirable to have a larger aperture to collect 'snake scattered' return photons and increase the signal. However, this may lead to an increase in the level of stray ambient light, particularly if a reasonably high level of illumination was required in the clinical examination environment. This effect may be especially important where extra fibres are used to increase the amount of signal to the photodetector, as in the embodiments of the present invention shown in figures 5 to 8.

This effect, in both endoscopes and microscopes (including endomicroscopes), may be reduced by enclosing the head or objective lens in a sheath or other cover, surrounding or

substantially sealable against the tissue being examined. If ambient light is still detected, however, this effect may be further reduced or eliminated by providing the general or ambient illumination from a light source which only produces light during the flyback period of the system, such as a strobe type system. In extreme circumstances the PMT voltages could also be cut during the flyback illumination period to avoid PMT saturation and overload.

Alternatively, or in addition, the room illumination may be light of one colour or range of colours, and the PMT provided with a filter that does not significantly transmit that colour or colours. Thus, for example, the source of ambient illumination could be a red light source, or a light source behind a red filter, and the PMT provided with a blue or blue/green filter.

Further, in order to reduce the levels of light impinging the PMT during non-acquisition periods, any of these techniques may be supplemented by means of a shutter (such as a mechanical shutter, or an electro-optical shutter) over the PMT. This shutter would be closed during non-acquisition periods (to protect the PMT from ambient light that might contribute to noise levels) but open during acquisition periods.

Figure 11A is a graphical representation of the varying position of the scanning mirror of an endomicroscope. The scan position varies essentially as a sine wave 280 with (in this example) a frequency of 750 Hz.

Image acquisition periods 282 and 284 are shown in figure 11B, with their timing relative to the mirror scan position indicated by dashed lines 282a and 284a.

Thus, ambient illumination is restricted to those periods when no acquisition occurs, as shown in figure 11C as shaded regions 286 and 288.

In some particular applications it may be desirable or necessary to employ high beam powers, such as in deep tissue imaging up to 500 mW may be desired. However, non-linear optical effects in the glass of the fibre, due to self phase modulation, can become a serious source of power loss and short wavelength generation at peak powers above approximately 50 mW at a 100 fs pulse rate in a standard optic fibre.

This problem may be reduced according to the present invention in several ways.

The first of these arrangements is illustrated in figure 12, which depicts an endomicroscope 290 similar to that shown in figure 4. It is to be understood, however, that this and the further arrangements described below may be employed with endomicroscopes according to any of the embodiments of the present invention.

Referring to figure 12, an endomicroscope 290 includes a laser source 292, two sets of prisms 294 and 296, focusing lens 298, first optic fibre 300, biconical taper coupler 302, fibres 304 and 306 including, respectively, reverse chirped Bragg grating fibres 308 and 310 oriented with the shorter spaced grating elements nearer the coupler 302 than the longest space grating elements, and endomicroscope head 312, optically connected to coupler 302 by second optic fibre 314. Figure 12 illustrates the head 312 located for examination of a tissue sample 316. Fluorescent light from the tissue sample 316 is detected by photo multiplied tube 318 after passing through BG39 spectral filter 320.

Endomicroscope 290 operates similarly to endomicroscope 160 of figure 4. However, endomicroscope 290 additionally includes a dispersion means in the form of first block of high dispersion glass 322 located between laser source 292 and prism pair 294, and compression means in the form of a second block of high dispersion glass 324 located within

endomicroscope head 312, between first lens 326 and second lens 328. The length of first and second optic fibres 300 and 314 are reduced to allow for the additional dispersion effects introduced by first and second glass blocks 322 and 324.

When the pulsed beam from laser source 292 passes through first glass block 322, the beam is subjected to an initial dispersion before entering first optic fibre 300. Consequently, the peak power of the beam is reduced within first optic fibre 300, relative to its value if glass block 322 were not employed, and consequently non-linear optical effects within the glass of first and second optic fibres 300 and 314 are reduced.

Unlike in endomicroscope 160, in which the recompression of the beam is virtually complete by the time the beam exits second optic fibre 182, in endomicroscope 290 the beam exists second optic fibre 314 without completed recompression, so that the peak power within second optic fibre 314 remains at a reduced level. The second glass block 324 provides the additional required recompression, only after the beam has exited the second optic fibre 314 and the problem of non-linear optical effects within the second fibre 314 have been avoided.

The glass blocks 322 and 324 will typically be high dispersion lead glass, but other highly dispersive elements, including gratings or other forms of prism, are also suitable.

As noted above, the length of first and second optic fibres 300 and 314 are reduced to compensate for the introduction of glass blocks 322 and 324. It should be noted, however, that the length of glass blocks 322 and 324 need not be equal: any inequality in their lengths can be compensated for either by adjusting the individual lengths of first optic fibre 300 and/or second optic fibre 314, or by adjusting the gratings 308 and 310. It may be highly

desirable in some applications that the length of second glass block 324 is minimised, so that minimal weight and length is added to endomicroscope head 312. Indeed, it may be acceptable or desirable in some applications to omit first glass block 322 entirely.

A second arrangement according to the present invention by which the problem of non-linear optical effects within the optic fibres may be reduced, is to use single moded fibre with a large diameter core for the first and second optic fibres (for example, fibres 168 and 182 in the embodiment of figure 4, or fibres 300 and 314 in the embodiment of figure 12). The first and second fibres would therefore have low v numbers and hence effectively low NAs. In principle, such single moded, large diameter core fibres may also be employed between the coupler and the Bragg gratings, but - as at this point in the optical path the beam is highly dispersed - this is not essential.

The areal power density within the core is therefore reduced, eliminating the problem of non-linear effects within the core of the first and second optic fibres. The disadvantage with this arrangement is that - owing to the larger diameter of the optic fibres - only more gradual curves can be created in the fibre. However, in some applications this may not create any inconvenience.

A third method for reducing the problem of non-linear optical effects in the optic fibres would be to use optic fibres with pure silica cores and fluoride doped cladding, in which fibres non-linear effects are reduced compared with conventional GeO_2 doped cores with pure silica cladding.

Finally, a combination of each of these techniques may be used. For example, a pair of particularly short glass blocks may be used, in the manner illustrated with endomicroscope 290 of figure 12, the length of the blocks being kept to a minimum for space and weight

considerations, but with large diameter core single moded optic fibres used for the first and second optic fibres. The combined effect of these two modifications can then be used to keep the peak power of the beam within the first and second optic fibres to an acceptable level.

Modifications within the spirit and scope of the invention may readily be effected by persons skilled in the art. It is to be understood, therefore, that this invention is not limited to the particular embodiments described by way of example hereinabove.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An endoscope or microscope including:

an optic fibre for transmitting a pulsed main beam along at least a portion of an optical path between a beam source and a tissue or other sample; and

a dispersing means;

whereby dispersion occurring in said optic fibre is reversed by dispersion produced by said dispersing means.

2. An endoscope or microscope as claimed in claim 1, wherein said dispersing means includes a compression means located optically after the optic fibre, for compressing the beam after the beam leaves the optic fibre, to reduce the peak power of the main beam within the optic fibre.

3. An endoscope or microscope as claimed in either claim 1 or 2, including a dispersion means located optically before said optic fibre, for dispersing said main beam before said main beam enters said optic fibre.

4. An endoscope or microscope as claimed in any one of the preceding claims, wherein said optic fibre has a large diameter core and hence low v number, to reduce areal power density in said core.

5. An endoscope or microscope as claimed in any one of the preceding claims, wherein said optic fibre has a pure silica core and fluoride doped cladding, to minimise non-linear optical effects within said fibre.

6. An endoscope or microscope as claimed in any one of the preceding claims, wherein said dispersing means includes a further optic fibre.

7. A method of two photon endoscopy or microscopy including the steps of:

transmitting a pulsed main laser beam along an optic fibre along at least a portion of an optical path between a

beam source and a tissue or other sample; and

dispersing said beam by means of dispersing means;

whereby dispersion occurring in said optic fibre is reversed by dispersion produced by said dispersing means.

8. An endoscope or microscope including:

a beam splitter means for splitting a pulsed main laser beam into first and second beams;

a first optic fibre for transmitting said main beam to said beam splitter means;

an antidispersive means for reducing the dispersion of said first and second beams;

an optical coupler means for recombining at least a portion of each of said first and second beams into a recombined beam; and

a second optic fibre for transmitting said recombined beam from said optical coupler means to an endoscope or microscope head;

wherein said main beam may be split by said beam splitter means into said first and second beams, whose dispersion may then be reduced by said antidispersive means, and then recombined by said optical coupler means to form a recombined two photon beam for transmission along said second optic fibre to said head.

9. An endoscope or microscope as claimed in claim 8, wherein said endoscope or microscope includes a laser source to provide said main beam.

10. An endoscope or microscope as claimed in claim 9, wherein said laser source is an ultrashort pulse laser.

11. An endoscope or microscope as claimed in any one of claims 8 to 10, wherein said first optical fibre is single moded at the wavelength of said main beam.

12. An endoscope or microscope as claimed in any one of claims 8 to 11, wherein each of said beam splitter means and/or said optical coupler means is an optical coupler.

13. An endoscope or microscope as claimed in any one of claims 8 to 11, wherein said beam splitter means and said optical coupler means are provided by a single optical coupler.

14. An endoscope or microscope as claimed in any one of claims 8 to 13, wherein said beam splitter means divides said main beam approximately equally into said first and second beams.

15. An endoscope or microscope as claimed in any one of claims 8 to 14, wherein said antidispersive means includes a reverse chirped Bragg grating fibre with more closely spaced gratings located optically closer to said beam splitter means.

16. An endoscope or microscope as claimed in any one of claims 8 to 14, wherein said antidispersive means includes first and second reverse chirped Bragg grating fibres, one for receiving each of said first and second beams, each with more closely spaced gratings located optically closer to said beam splitter means.

17. An endoscope or microscope as claimed in any one of claims 8 to 16, wherein said endoscope or microscope further includes a large diameter optic fibre and a cladding mode coupler, wherein said cladding mode coupler couples said second optic fibre and said large diameter fibre so that large angle scattered light from said head will be substantially coupled into said large diameter fibre by said coupler for transmission to a photodetector.

18. An endoscope or microscope as claimed in any one of claims 8 to 17, wherein said endoscope or microscope includes a compression means located optically after said second optic fibre, for compressing said recombined beam after said recombined beam leaves said second optic fibre, whereby the peak power of said main beam within said first

optic fibre and of said recombined beam in said second optic fibre is reduced.

19. An endoscope or microscope including:

a dispersing means for temporally dispersing a pulsed main laser beam spectroscopically; and

an optic fibre for transmitting said dispersed main beam to an endoscope or microscope head and reducing said dispersion by compressing said dispersed main beam.

20. An endoscope or microscope as claimed in claim 19, wherein said optic fibre substantially reverses the dispersion introduced by the dispersing means.

21. An endoscope or microscope as claimed in claim 19, wherein said endoscope or microscope has a head and said head includes a compression means located optically after said optic fibre, for furthering or completing said compression of the dispersed main beam, to reduce the peak power of the main beam within the optic fibre.

22. An endoscope or microscope as claimed in any one of claims 19 to 21, wherein said dispersing means is also a beam splitter means for selecting the fluorescent light emitted by a tissue or other sample.

23. An endoscope or microscope as claimed in any one of claims 19 to 22, wherein said dispersing means includes a pair of diffraction or refraction elements.

24. An endoscope or microscope as claimed in any one of claims 19 to 23, wherein said dispersing means includes a pair of blazed gratings and a prism.

25. An endoscope or microscope with a head, said endoscope or microscope including:

a suction or partial vacuum source located near said head;

a light collecting means for collecting light from a

sample; and

a sample receiving means coupled to said suction source for receiving said sample when said sample is urged thereto by said suction source;

wherein said suction source is used to urge said sample towards said receiving means, thereby deforming said sample so that said light collecting means can collect some of light emitted by said sample away from said head.

26. An endoscope or microscope as claimed in claim 25, wherein said sample receiving means is cup shaped or frustum shaped.

27. An endoscope or microscope as claimed in either claim 25 or 26, wherein said light collecting means is, in use, separated from said sample by said sample receiving means, and said sample receiving means is substantially transparent to said light between said sample and said light collecting means.

28. An endoscope or microscope as claimed in any one of claims 25 to 27, wherein said light collecting means comprises an optic fibre.

29. An endoscope or microscope as claimed in any one of claims 25 to 28, wherein said light collecting means comprises a mirror.

30. An endoscope or microscope as claimed in claim 29, including a lens for focussing light from said mirror.

31. An endoscope or microscope as claimed in any one of claims 25 to 30, wherein said light collecting means is one of a plurality of light collecting means.

32. A method of two photon endoscopy or microscopy including the steps of:

1) transmitting a pulsed main laser beam along an optic fibre;

2) splitting said main beam into first and second beams;

3) compressing said first and second beams;

4) combining at least a portion of said first and second beams;

5) transmitting said first and second beams along an optic fibre to an endoscope or microscope head.

33. An endoscope or microscope system including:

an endoscope or microscope, including a photodetector;

an illumination means for providing ambient illumination;

switching means for switching said illumination means on and off; and

synchronization means for synchronizing said switching means with acquisition periods of said endoscope or microscope;

wherein said synchronization means is operable to control said switching means to switch said illumination means off during said acquisition periods and on between consecutive acquisition periods.

34. An endoscope or microscope system as claimed in claim 33, wherein said synchronisation means is operable to control said switching means to switch said illumination means such that said illumination means is on during the flyback period of said endoscope or said microscope.

35. An endoscope or microscope system as claimed in either claim 33 or 34, wherein said photodetector is operable to be switched off when said illumination means is on.

34. An endoscope or microscope system as claimed in any one of claims 33 to 35, wherein said photodetector has a power supply operable to be switched off when said illumination means is on.

37. An endoscope or microscope system including:

an endoscope or microscope, including a photodetector;

an illumination means for providing ambient illumination; and

filter means for reducing the detection of said ambient illumination by said photodetector.

38. An endoscope or microscope system as claimed in claim 37, wherein said illumination means emits light preferentially blocked by said filter means.

39. An endoscope or microscope system as claimed in claim 37, wherein said filter means is a first filter means, and the system includes a second filter means for absorbing light from said illumination means that is preferentially passed by said first filter means and transmitting light from said illumination means that is preferentially blocked by said filter means.

40. An endoscope or microscope system as claimed in any one of claims 37 to 39, including switching means for switching said illumination means on and off, and synchronization means for synchronizing said switching means with acquisition periods of said endoscope or microscope, wherein said synchronization means is operable to control said switching means to switch said illumination means off during said acquisition periods and on between consecutive acquisition periods.

41. An endoscope or microscope system as claimed in any one of claims 37 to 40, including a shutter for occluding said photodetector, wherein said synchronization means is operable to control said shutter means to occlude said photodetector when said illumination means is on, and expose said photodetector during said acquisition periods.

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34

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Application No: GB 9914002.2 **Examiner:** Chris Ross
Claims searched: 1-24 **Date of search:** 6 September 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): G2J(JB7R4)

Int Cl (Ed.6): G02B

Other: Online: WPI, EPODOC, JAPIO

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
	None	

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